REMARKS

The Office Action

Claims 30-47 and 50-62 are pending in this application, but claims 32, 35, 38-42, and 45 are withdrawn from consideration. All examined claims stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. Claims 30-31, 33-34, 37, 43-44, 47, and 50-53 stand further rejected under 35 U.S.C. § 102 as anticipated by either Tsukamoto *et al.* (*J. Clin. Invest.* 100:107-114, 1997; "Tsukamoto"), in light of Breslow *et al.* (*J. Biol. Chem.* 257:14639-14641, 1982; "Breslow"). Claims 30-31, 33-34, 36-37, 43-44, 47, and 50-53 stand further rejected under 35 U.S.C. § 102 as anticipated by Kashyap *et al.* (*J. Clin. Invest.* 96:1612-1620, 1995; "Kashyap"), as evidenced by Breslow. Claims 46 and 56-62 are objected to. Each of these objections and rejections are address individually below.

Objections

The Examiner objects to the use of the abbreviation "LDL" in claim 46. Claim 46 has been amended in accordance with the Examiner's suggestion and this objection may be withdrawn.

The Examiner objects to claims 56-62 as containing non-elected species. Applicants respectfully note that claims 56-62 encompassing several specific variants of the ApoE gene. As elected in Applicants' Reply to Restriction Requirement (submitted January 2, 2003), Applicants request that these claims be examined to the extent of the elected ApoE species, ApoE3 (SEQ ID NO: 15). In the event that the generic claim (claim 30) is found allowable, the dependent claims to elected and unelected species encompassed by that generic claim must also be allowable. 37 C.F.R. § 1.141; M.P.E.P. § 809.04. It is unclear to Applicants what correction is required.

Rejections Under 35 U.S.C. § 112, first paragraph

All pending claims stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner asserts that, while being enabling for a method of lowering cholesterol by intravascular administration of the identified recombinant adenovirus to a mammal lacking a normally functioning ApoE gene, the specification does not enable a method for lowering cholesterol, delaying the onset of atherosclerosis, or regressing atherosclerosis in any mammal by administering the adenovirus by any route or causing protein expression in any tissue. Specifically, the Examiner argues that the specification provides insufficient guidance on the use of gene therapy generally and the use of recombinant ApoE fragments in a manner that does not induce hypertriglyceridemia. The rejection is based on (i) the unpredictability of the duration of gene expression, (ii) a lack of cholesterol-lowering effect of the ApoE fragments in "normal" mammals, (iii) the hypertriglyceridemia-inducing effects of the ApoE fragments, (iv) the criticality of ApoE fragment levels with specific regard to a gene dosage effect, and (v) the unpredictability of tissue targeting of the expression vector. Each of these bases of rejection are addressed individually below.

General Unpredictability of the Art

The Examiner asserts that, as of the effective filing date of the application, "the state of the gene therapy art remains unpredictable particularly for the attainment of the desired prophylactic and/or therapeutic effects" (*Office Action*, page 6). In support of unpredicability, the Examiner cites numerous prior art publications as evidence that gene therapy.

As an initial matter, none of the references cited by the Examiner suggest that gene therapy does not work or is completely unpredictable. The references merely suggest that gene therapy has not been optimized sufficiently for certain (but not all) therapeutic ends. For example, Dang *et al.* (*Clin. Cancer Res.* 5: 471-474, 1999; cited by the Examiner)

notes that there are about 300 approved gene therapy clinical trials currently underway worldwide. Dang *et al.* and Verma *et al.* (Nature 389: 239-242, 1997; cited by the Examiner) both note that many of the clinical trials are directed to chronic diseases requiring long-term therapy such as cancer, AIDS, and neurodegenerative disease. Dang *et al.* further note that, in one AIDS patient transduced with the ADA gene, ADA cDNA was produced by leukocytes for at least four years after the gene therapy was administered. Although the clinical outcome following the cessation of other anti-AIDS therapies was not successful, Dang *et al.* point out that this example serves as a scientific "proof of principle" (*Dang* at page 471). The results disclosed in the prior art are not evidence that gene therapy does not work. They are merely evidence that optimization of gene therapy, requiring no more than routine experimentation, is needed.

Duration of Gene Expression

The Examiner, citing Dang *et al.*, asserts that long term stable expression using gene therapy techniques is unpredictable. Dang *et al.* suggest that over half of the currently approved gene therapy trials are for the treatment of cancer and many more for the treatment of genetic diseases or complex non-genetic diseases like AIDS. Applicants point out that a commonality among the traditional diseases targeted using gene therapy is the requirement for long, possibly indefinite, durations of gene expression.

Applicants respectfully direct the Examiner's attention to Verma *et al.* who confirm Applicants' teachings that a wide variety of vectors suitable for gene therapy are available, each having different advantages and disadvantages. Verma *et al.* review the characteristics of retroviral, lentiviral, adenoviral, adeno-associated viral, and other, less widely used vectors (e.g., herpes simples, vaccinia-virus, Sindbis, and Semliki Forest virus vectors). With respect to the adenoviral vectors (the elected species in the instant application), Verma *et al.* teach that these vectors have been used successfully to infect cells *in vivo*, resulting in very high expression levels of the transgene (page 241, col. 1,

second paragraph). The disadvantage of the adenoviral vectors, according to Verma *et al.*, is the relatively short duration of expression. Verma *et al.* discloses that typical expression durations are 5-10 days, 20-40 days, and at least 84 days for first generation, second generation, and "gut-less" adenoviral vectors, respectively (page 241, cols. 1-2). These observations lead Verma *et al.* to conclude that "adenoviral vectors are extremely useful if expression of the transgene is required for short periods of time" (page 241, col. 2, first paragraph; emphasis added).

Applicants also respectfully direct the Examiner's attention to Chen *et al.* (*Mol. Ther.* 2: 256-261, 2000) and Oka *et al.* (*Circulation* 103: 1274-1281, March 6, 2001). Both references demonstrate that transgene expression is detectable in the liver for at least six months following infection using "gut-less" adenoviral vectors. Furthermore, the expression of an anti-hypercholesterolemic protein (the VLDL receptor) was of a sufficient level and a sufficient duration to effect a significant and prolonged reduction in plasma cholesterol—the same clinical outcome as for the presently claimed invention. The anti-hypercholesterolemic effect of transgene expression was measurable as early as 7 days after infection (Oka *et al.*, Figure 4).

Applicants' method, unlike many gene therapy trials in the prior art, may not require prolonged periods of transgene expression. By contrast, Applicants' invention provide a method for lowering cholesterol, a condition that, if treated using gene therapy, does not necessarily require long-term expression of the ApoE fragment. The temporary expression of ApoE fragments may be sufficient to provide immediate reduction of plasma cholesterol; a condition that may be subsequently maintained by existing drug therapies, dietary modification, or re-injection of a vector of the invention. Accordingly, the unpredictable nature of long-term expression using gene therapy techniques is not a proper basis to support a lack of enablement rejection.

The Cholesterol-lowering Effects of ApoE Fragments

The Examiner disagrees with Applicants' interpretation of the data – that expression of an ApoE fragment reduces cholesterol levels – and its applicability to mammals that are not deficient in endogenous ApoE. Specifically, the Examiner states:

> [T]he specification teaches specifically that... the overexpression of apoE4-202 in normal mice increased the cholesterol levels of normal mice. (*Office Action*, page 8; emphasis original)

This interpretation is not accurate. Applicants point out that any perceived difference between the AdGFP-E3 animals at t=0 and the AdGFP-E4-202 animals (Figure 16A) at any time is nominal. The difference is not statistically significant, falling into the normal variability associated with measuring a small amount of blood cholesterol. Certainly, the plasma cholesterol levels are significantly lower than those measure in wildtype animals overexpressing full length apoE3 or apoE4.

Applicants also direct the Examiner's attention to Figure 17—showing the cholesterol and triglyceride levels of individual animals. Figure 17C demonstrates that plasma cholesterol levels in normal C57BL6 mice are not elevated by overexpression of apoE4-202 compared to apoE$^{-/-}$ mice that overexpress a truncated apoE4, which represent normal plasma cholesterol levels.

Applicants' assertion—that overexpression of apoE4-202 in apoE-expressing mammals does not increase plasma cholesterol—has recently been validated (Gerritsen *et al. J. Lipid Res.* 44:408-418, 2003; copy enclosed). Gerritsen *et al.* express apoE4-202, using an adenoviral vector, in ApoE$^{-/-}$ mice that are transgenic for human apoE2 (APOE2.*Apoe*$^{-/-}$ mice). Figure 1C demonstrates that expression of apoE4-202 does not cause a hypercholesterolemic phenotype in mice expressing human apoE2. Thus, contrary to the Examiner's assertion, cholesterol levels in are not increased in mammals expressing a functional, full-length apoE gene.

The Hypertriglyceridemia-inducing Effects of ApoE Overexpression

The Examiner questions Applicants' findings – that overexpression of the disclosed apoE4 fragments does not induce hypertriglyceridemia – by relying on the teachings of Dijk *et al.* (*J. Lipid Res.* 40: 336-344, 1999). The Examiner points out that expression of full length apoE3 in LDL receptor-deficient mice of Dijk *et al.* induces hypertriglyceridemia. In this regard, the teachings of Dijk *et al.* have no bearing on this particular issue because Dijk *et al.* use full length apoE proteins, whereas Applicants' invention uses apoE fragments. Applicants' invention is based on the discovery that the hypertriglyceridemia-inducing effects of apoE can be dissociated from the cholesterol-reducing effects by truncating the apoE protein. However, in so far as Dijk *et al.* prove that over-expression of a full length apoE induces hypertriglyceridemia, the findings are in accordance with Applicants' specification and data (Figure 16B – C57BL6 mice). The teachings of Dijk *et al.* are not inconsistent with Applicants' data.

The Criticality of ApoE Levels – Gene Dosage Effects

The Examiner further asserts that the specification does not provide sufficient guidance for maintaining therapeutic and/or prophylactic apoE levels. This aspect of the rejection is based on Dijk *et al.* which demonstrates that plasma apoE levels delivered by adenovirus-mediated gene transfer, declined rapidly after 14 days and Mahley *et al.* which suggests that "a rather narrow range of apoE levels is required to maintain normal plasma lipid levels." The Examiner concludes that

> gene dosage and expression levels of apoE are critical for the attainment of prophylactic and/or therapeutic effects without the induction of hypertriglyceridemia as contemplated by Applicants. (*Office action*, paragraph bridging pages 8-9).

With regard to the teachings of Dijk *et al.*—that apoE levels decline rapidly 14

days after adenoviral-mediated gene transfer, Applicants again emphasize that adenoviral vectors other than the type used by Dijk *et al.* were known, at the time of application filing, to result in longer duration gene expression. As discussed above, Applicants again point out that vector-mediated expression of another anti-hypercholesterolemic protein (the VLDL receptor) reduced plasma cholesterol levels in as little as seven days after infection (Oka *et al., supra*). Furthermore, short duration gene expression may be a desirable property when used in conjunction with dietary and lifestyle modifications to augment the cholesterol-lowering effects of the apoE fragments.

Turning next to Mahley *et al.*, while it may be true that the beneficial effects of elevated levels of <u>full-length</u> apoE (lower cholesterol without elevating triglycerides) occur only in a narrow therapeutic window, Applicants stress that the present method uses apoE <u>fragments</u>. The present invention is based on Applicants' discovery that the cholesterol-lowering activity of apoE is dissociable from the activity that causes hypertriglyceridemia. Specifically, the triglyceride-inducing activity is abolished by truncating the C-terminus of the apoE proteins, rendering the fragments superior to the full-length protein.

Applicants clearly demonstrate that the apoE fragments of the present invention have a wide therapeutic window. For example, cholesterol levels are reduced by approximately 75% as early as four days after administration of $2x10^9$ pfu of the adenoviral vector encoding apoE4-202 (Figure 3B), whereas no significant elevation in triglyceride levels was measured following administration of as much as $1x10^{10}$ pfu; a 5-fold excess (Figure 3A). By contrast, administration of $2x10^9$ pfu of the adenoviral vector encoding the full length apoE4 resulted in approximately 15-20% reduction in cholesterol and 10-fold increase in triglyceride levels (see Figure 4A).

Taken together, the available data demonstrates that the skilled artisan can expect a wide range of concentrations of the apoE fragments of the invention to be effective in the presently claimed method.

Tissue Targeting of the Vector and the Unpredictability of the Physiological Art

The Examiner further asserts that

> [v]ector targeting *in vivo* to desired tissues or organs (for this instance to the liver) continues to be unpredictable and inefficient. This is supported by numerous teaching available in the art as well as the aforementioned review articles (citations omitted). (*Office Action*, page 10).

Applicants respectfully direct the Examiner's attention to Chen *et al.* (*Mol. Ther.* 2: 256-261, 2000) and Oka *et al.* (*Circulation* 103: 1274-1281, March 6, 2001). These references demonstrate that transgene expression in the liver was detected for at least six months following infection using adenoviral vectors. Although there is no indication whether this expression is "liver-specific," tissue-specific expression is not required. Both studies further demonstrate expression of a therapeutic protein for sufficient, and limited, duration to effect a reduction in plasma cholesterol and regression of atherosclerotic plaques—the same clinical endpoints as for the present invention. Because tissue-specific expression of the apoE fragment is required neither for a successful clinical outcome nor in the presently claimed method, it is an inappropriate basis to support a lack of enablement rejection.

In view of the foregoing, Applicants respectfully submit that the present specification, in combination with the prior art, is sufficient to allow a skilled artisan to practice the claimed invention using no more than routine experimentation. Applicants request that this rejection be withdrawn.

Rejections Under 35 U.S.C. § 102(b)

Claims 30-31, 33-34, 37, 43-44, 47, and 50-53 stand rejected under 35 U.S.C. § 102(b) as anticipated by Tsukamoto *et al.* (*J. Clin. Invest.* 100:107-114, 1997; "Tsukamoto"), as evidenced by Breslow *et al.* (*J. Biol. Chem.* 257:14639-14641, 1982; "Breslow"). Specifically, the Examiner points out that Tsukamoto discloses the infection of ApoE-deficient mice with a recombinant adenovirus capable of expressing a mature human ApoE3 having 299 amino acids. The Examiner asserts that this infection resulted in a reduction in the plasma total cholesterol level and a small but significant reduction in plasma triglyceride levels, demonstrating no induction of hypertriglyceridemia.

Claims 30-31, 33-34, 36-37, 43-44, 47, and 50-53 stand further rejected under 35 U.S.C. § 102(b) as anticipated by Kashyap *et al.* (*J. Clin Invest.* 96:1612-1620, 1995; "Kashyap"), as evidenced by Breslow. The Examiner asserts that Kashyap discloses the treatment of ApoE-deficient mice using recombinant adenovirus expressing full-length mature human ApoE3 having 299 amino acids. The Examiner further asserts that Kashyap demonstrates normalization of the lipid and lipoprotein profile including reduced cholesterol, VLDL, IDL, and LDL, and increased HDL levels.

Applicants point out that the claims, as presently amended, are limited to the use of nucleic acids encoding polypeptides having fewer than 299 amino acids. Neither Tsukamoto nor Kashyap teach or suggest using a truncated ApoE protein.

Furthermore, the results presented in Tsukamoto and Kashyap are not necessarily typical of the biological activity attributed to the ApoE proteins at the time of application filing. Applicants respectfully direct the Examiner's attention to the specification at page 6, lines 4-25, and references cited therein. At the time of application filing, evidence existed which suggested that ApoE functioned to decrease cholesterol while increasing triglyceride levels. See, for example, Huang *et al., J. Biol. Chem.* 273:26388-26393, 1998 (demonstrating that "expressing high plasma levels of human apoE3 in transgenic mice lacking endogenous mouse apoE caused [hypertriglyceridemia]" (abstract)); and

Cohn *et al., Arterioscler. Thromb. Vas. Biol.* 16:149-159, 1996 (Table 3 demonstrates a positive correlation between total plasma ApoE of all phenotypes with total plasma triglyceride concentration suggesting that elevated ApoE levels causes hypertriglyceridemia); each of which are art of record. Thus, the art strongly suggests that full length ApoE possess a hypertriglyceridemia-inducing properties.

Applicants data is consistent with the hypertriglyceridemic effects of ApoE overexpression most prevalent in the prior art. Figures 5E-F, 8A, 10A-B, for example, demonstrate that overexpression of full length ApoE4 causes a marked increase in triglyceride levels; an effect not caused by similar expression of the ApoE4-202, ApoE4-229, or ApoE4-259 truncations. Applicants demonstrate in the accompanying figures, through systematic C-terminal truncation, that the cholesterol-lowering effect of the ApoE proteins may be dissociated from the hypertriglyceridemia -inducing effect.

Thus, while Tsukamoto and Kashyap may suggest that a full length ApoE protein is capable of reducing plasma cholesterol without inducing hypertriglyceridemia (contrary to much of the scientific evidence available at the time of application filing), nothing in those references suggest using an ApoE truncation, as presently claimed by Applicants. Accordingly, in view of the present amendments, these rejections should be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is requested. Enclosed is a petition to extend the period for replying for two months, to and including January 20, 2004. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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